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Interpretive Summary
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Improving the conversion of feed into milk and body tissues in dairy cattle is important for economic and environmental sustainability of the dairy industry. There is a genetic basis to the utilization of feed by the dairy cow, but underlying genes that contribute to the trait are not well identified. Results of this study suggest that many genes, each with a small effect, impact feed efficiency and the genetic basis of feed efficiency varies with parity. Also, chromosomal regions and candidate genes related to feed efficiency and other relevant biologically and economically important traits are identified.

The genetic and biological basis of feed efficiency in mid-lactation Holstein dairy cows

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ABSTRACT

The objective of this study was to identify genomic regions and candidate genes associated with feed efficiency in lactating Holstein cows. In total, 4,916 cows with actual or imputed genotypes for 60,671 SNP having individual feed intake, milk yield, milk composition, and body weight records were used in this study. Cows were from research herds located in the US, Canada, the Netherlands, and the United Kingdom. Feed efficiency defined as residual feed intake (RFI) was calculated within location as the residual of the regression of dry matter intake (DMI) on milk energy (MilkE), metabolic body weight (MBW), change in body weight, and systematic effects. For RFI, DMI, MilkE, and MBW, bivariate analyses were performed considering each trait as a separate trait within parity group in order to estimate variance components and genetic correlations between them. Animal relationships were established using a genomic relationship matrix. Genome-wide association studies were performed separately by parity group for RFI, DMI, MilkE, and MBW using the Bayes B method with a prior assumption that 1% of SNP have a non-zero effect. One megabase (Mb) windows with greatest percentage of the total genetic variation explained by the markers (TGVM) were identified, and adjacent windows with large proportion of the TGVM were combined and reanalyzed. Heritability estimates for RFI were 0.14 ($\pm$ 0.03) in primiparous cows and 0.13 ($\pm$ 0.03) in multiparous cows. Genetic correlations between primiparous and multiparous cows were 0.76 for RFI, 0.78 for DMI, 0.92 for MBW, and 0.61 for MilkE. No single 1-Mb window explained a significant proportion of the TGVM for RFI; however, analyses identified adjacent regions explaining the greatest percentage of the TGVM on BTA 27 in primiparous cows and on BTA 4 in multiparous cows. Candidate genes in these regions include *beta-3 adrenergic receptor* and *leptin*, respectively. Between the 2 parity groups, 3 of the 10 windows with large effects on DMI

neighbored windows with greatest effects on RFI, but were not in the top 10 regions for MilkE or MBW. This result suggests there is a genetic basis for feed intake that is unrelated to energy consumption required for milk production or expected maintenance as determined by MBW. In conclusion, feed efficiency measured as RFI is a polygenic trait exhibiting a dynamic genetic basis and genetic variation distinct from that underlying expected maintenance requirements and milk energy output.

Key words: GWAS, residual feed intake, feed efficiency, dairy

INTRODUCTION

Improvement in feed efficiency in dairy cattle is important in that it results in reduced greenhouse gas emissions (Knapp et al., 2014), less land and resources needed for the production of feed (von Keyserlingk et al., 2013), and economic benefits through reduced inputs for equivalent output, as feed represents more than 50% of the total cost of producing milk (USDA-NASS, 2015). Over the past 100 years, cows have become more feed efficient largely through increases in milk production, thereby diluting the proportion of feed used for maintenance (VandeHaar and St-Pierre, 2006). However, because this effect diminishes with each successive incremental in production relative to body size, continued gains via this route are diminishing (Vandehaar et al, 2016), warranting the exploration of the genetic basis of feed utilization in lactating dairy cattle for targeted selection.

Identifying genetically superior animals for feed efficiency is a difficult task that requires many animals with phenotypes in order to accurately predict an animal's genetic merit for feed efficiency. Thus, large collaborations between European, North American, and Australasian researchers have been established in order to pool feed intake data (Berry et al., 2014; de Haas et al., 2015; Tempelman et al., 2015). In one collaboration, nearly 5,000 cows have been

genotyped and phenotyped for feed intake and related traits (Tempelman et al., 2015; Vandehaar et al., 2016). Specifically, these cows have phenotypes for residual feed intake (**RFI**), which is defined as the actual intake minus the intake that is expected based on level of production and animal size (Koch et al., 1963). In mid-lactation dairy cows, RFI is often computed as the residual of the regression of intake on a form of energy-corrected milk production, metabolic body weight (**MBW**), and energy gained or lost in body tissues. Tempelman et al. (2015) estimated RFI to have a heritability of 0.15 to 0.18 in this population, suggesting a genetic basis to RFI.

Presently, a limited number of genome-wide association studies (**GWAS**) have been performed in order to identify QTL and subsequently candidate genes related to feed efficiency traits in dairy cattle. These studies have either utilized relatively small populations with limited power to detect QTL (Verbyla et al., 2010; Yao et al., 2013) or investigated the genetic architecture of feed efficiency in non-lactating heifers (Pryce et al., 2012) or only primiparous cows (Veerkamp et al., 2012; Tolkamp et al., 2014). However, biological mechanisms underlying variation in feed efficiency in growing animals may not be the same as that for mature lactating animals (Spurlock and VandeHaar, 2013).

The first goal of this study was to identify genomic regions associated with RFI in lactating Holstein cows, and compare those regions to QTL influencing traits underlying RFI, including DMI, maintenance energy requirements, and milk energy output. The second goal was to identify potential candidate genes that are located within RFI QTL and known to function within physiological pathways relevant to feed efficiency. To that end, we utilized data from nearly 5,000 lactating Holstein cows to identify genomic regions and candidate genes associated

with RFI and related traits. Differences in the genetic basis of RFI associated with parity were also explored.

MATERIALS AND METHODS

Data Collection

For detailed information on the collection of phenotypes used in this project, see Tempelman et al. (2015). For the current study, phenotypes meeting the criteria outlined below were available on 6,453 cows from research stations within the United States, Canada, the Netherlands, and the United Kingdom. Records were very heterogeneous as described in Tempelman et al. (2015), but for each cow, most of the research stations provided daily feed intake and milk production, a minimum of starting and ending BW for the recording period and biweekly observations of milk fat, protein, and lactose percentages. Only measurements collected between 50 and 200 DIM were used because this is when the cow is at peak DMI, and BW is relatively stable.

Individual measurements were edited and then combined to form one 28-day average phenotype each for DMI, milk energy (**MilkE**; determined as the sum of the energy in the fat, protein, and lactose in the milk; NRC, 2001), MBW ($BW^{0.75}$), and change in BW (**ΔBW**). Phenotypes for RFI were calculated similarly to Tempelman et al. (2015) within location as the residual of the regression of DMI on MilkE, MBW, and ΔBW plus systematic effects:

$$DMI_{ijlm} = parity_i + \sum_{k=0}^{5} b_{ik} DIM_{ijlm}^{k} + \beta_1 MilkE_{ijlm} + \beta_2 MBW_{ijlm} + \beta_3 \Delta BW_{ijlm} + E_j + D_l(E_j) + T_m + RFI_{ijlm}$$

where $parity_i$ is the fixed effect of parity (primiparous or multiparous), $\sum_{k=0}^{5} b_{ik} DIM_{ijlm}^{k}$ is the 5th-order Legendre polynomial regression of DMI on DIM with parity-specific regression

coefficients b_{ik}, β_1 is the partial regression coefficient of DMI on MilkE, β_2 is the partial regression coefficient of DMI on MBW, β_3 is the partial regression coefficient of DMI on ΔBW, E_j is the fixed effect of experiment, $D_l(E_j)$ is the random effect of diet within experiment, T_m is the random effect of test date, and RFI_{ijlm} is the random error term and the phenotype used for RFI in further analyses. Test date was defined as the middle date of the window during which the cow had data recorded.

Genotypes were determined using various commercially available SNP chips, with the number of genotypes per cow ranging from 3K to 777K. All genotype data were processed by the Animal Genomics and Improvement Laboratory (AGIL, http://aipl.arsusda.gov; Wiggans et al., 2014). A final data set with genotypes for 60,671 SNPs for each animal was generated using imputation methods employed through the software findhap (http://aipl.arsusda.gov/software/findhap/). In total, 4,916 cows had genotypes and phenotypes for all traits, and each cow had up to one primiparous and one multiparous record used (Table 1). Therefore, 3075 primiparous records and 2667 multiparous records were used, and after imputation, these cows had 3.0 and 3.1 percent missing genotypes, respectively. Because a permanent environmental effect was not fitted, if a cow had multiple multiparous records, the parity used was randomly chosen.

Genetic Parameters

Variance components, heritabilities, and genetic correlations for each trait (RFI, DMI, MilkE, and MBW) between first and second or greater parities were estimated using bivariate analyses in ASReml 4.0 (Gilmour et al., 2015). For each trait, the phenotype measured during first parity was considered as trait one and the phenotype measured in a second or greater parity was considered trait two. While little to no culling based on feed efficiency was experienced in

the herds providing data, by using bivariate analyses, we accounted for any bias in variance component estimation that may have been due to culling. For DMI, MilkE, and MBW, within each trait, the following model was used:

$$y_{ijlmno} = \mu_i + \sum_{k=1}^{5} DIM^k_{ijlmno} + L_{ij} + D_m(E_l(L_j))_i + T_n(L_j)_i + g_{io} + \varepsilon_{ijlmno}$$

where parity-specific (primiparous or multiparous) fixed and random effects were denoted by subscript i, y_{ijlmno} is the observed DMI, MilkE, or MBW with overall mean μ_i, $\sum_{k=1}^{5} DIM^k_{ijlmno}$ is the 5th order Legendre polynomial regression of y on DIM, L_j is the fixed class effect of location (12 levels), $D_m(E_l(L_j))_i$ is the random effect of diet within experiment within location, $T_n(L_j)_i$ is the random effect of test date within location, g_{io} is the random genetic effect of animal, and ε_{ijlmno} is the random error. Random effects were assumed to follow multivariate normal distributions with mean equal to zero and covariance matrix:

$$\begin{bmatrix} \mathbf{u}_{DEL_1} \\ \mathbf{u}_{DEL_2} \\ \mathbf{u}_{TL_1} \\ \mathbf{u}_{TL_2} \\ \mathbf{g}_1 \\ \mathbf{g}_2 \\ \mathbf{e}_1 \\ \mathbf{e}_2 \end{bmatrix} = \begin{bmatrix} \mathbf{I}\sigma^2_{DEL_1} & \mathbf{I}\sigma_{DEL_1,DEL_2} & 0 & 0 & 0 & 0 & 0 & 0 \\ \mathbf{I}\sigma_{DEL_1,DEL_2} & \mathbf{I}\sigma^2_{DEL_2} & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & \mathbf{I}\sigma^2_{TL_1} & \mathbf{I}\sigma_{TL_1,TL_2} & 0 & 0 & 0 & 0 \\ 0 & 0 & \mathbf{I}\sigma_{TL_1,TL_2} & \mathbf{I}\sigma^2_{TL_2} & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & \mathbf{G}\sigma^2_{g_1} & \mathbf{G}\sigma_{g_1,g_2} & 0 & 0 \\ 0 & 0 & 0 & 0 & \mathbf{G}\sigma_{g_1,g_2} & \mathbf{G}\sigma^2_{g_2} & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & \mathbf{I}\sigma^2_{e_1} & \mathbf{I}\sigma_{e_1,e_2} \\ 0 & 0 & 0 & 0 & 0 & 0 & \mathbf{I}\sigma_{e_1,e_2} & \mathbf{I}\sigma^2_{e_2} \end{bmatrix}$$

where **I** denotes the identity matrix; **G** denotes the genomic relationship matrix that was constructed according to the first method of VanRaden (2008) using the 4,916 animals with phenotypes and genotypes; $\sigma^2_{DEL_i}$ denotes the variance component for the interaction of diet within experiment within location for parity group i with subscripts 1 and 2 denoting primiparous

and multiparous records, respectively; σ_{DEL_1,DEL_2} denotes the covariance between primiparous and multiparous cows for the interaction of diet within experiment within location; $\sigma^2_{TL_i}$ denotes the variance component for location specific effects of test dates for parity group *i* with σ_{TL_1,TL_2} denoting the covariance between parity groups for location specific effects of test dates; $\sigma^2_{g_i}$ denotes the animal polygenic variance for parity group *i* with σ_{g_1,g_2} denoting the polygenic covariance between parity groups; and $\sigma^2_{e_i}$ denotes the residual variance component for parity group *i* with σ_{e_1,e_2} denoting the residual covariance between the two parity groups. Because systematic effects were accounted for during calculation of RFI, only the animal effect was considered in the bivariate analysis between RFI estimated in primiparous and multiparous cows.

Genome-wide Association Analyses

Genome-wide association analyses were performed to identify QTL related to RFI, DMI, MBW, and MilkE using GenSel version 4.0 (Fernando and Garrick, 2009; Garrick and Fernando, 2013). Because the current version of GenSel does not accommodate random effects other than marker effects, adjusted phenotypes were calculated as the sum of the animal and error terms from univariate analyses according to the models described above. Method Bayes B was used to identify QTL using the following model:

$$y_i = \mu + \sum_{j=1}^{k} \delta_j m_{ij} \alpha_j + e_i$$

where y_i is the phenotype, μ is the overall mean, $\sum_{j=1}^{k} \delta_j m_{ij} \alpha_i$ is the genomic breeding value, modeled as the sum across *k* SNPs, with inclusion factor δ_j (coded 0 or 1 with prior probabilities π and 1-π, respectively, π set equal to 0.99), genotype *m* (coded as 0, 1, 2, or average for missing genotypes), allele substitution effect α_j for SNP *j*, and random error e_i. Method Bayes B assumes that the effect of

each SNP follows an independent, normal distribution with null mean and unknown SNP-specific variance. Therefore, the variance of each SNP is allowed to differ. All non-monomorphic SNP were used, and missing genotypes were replaced with the average genotype for that SNP (Boddicker et al., 2012). GenSel cannot accommodate missing values for SNP so by replacing the missing genotype with the mean genotype for that SNP, that genotype does not contribute to the estimate of the SNP effect. Priors for genetic and residual variances used in the above model were estimated using method Bayes C with all SNP included in the model ($\pi = 0$) (Habier et al., 2011). For this method, SNP effects are expected to follow a normal distribution with null mean and common variance σ_α^2 (Fernando and Garrick, 2013). For both BayesB and Bayes C Markov chain Monte Carlo (MCMC) sampling with a minimum of 120,000 iterations was used to estimate posterior means of SNP substitution effects with the first 20,000 iterations discarded. Convergence was assessed through visual inspection of the samples of the genetic variance.

The SNPs were binned into non-overlapping 1-Mb windows according to the UMD 3.1 map of the Bos taurus genome (http://bovinegenome.org/; Genbank accession: DAAA00000000.2), and the proportion of genetic variation explained by each window was estimated following Wolc et al. (2012). Under a pure polygenic model, it was assumed that each 1-Mb window explained an equal amount of the total genetic variance. Thus, the bovine genome was divided into 2,676 1-Mb windows, such that the expected percent of the total genetic variation explained by the markers (**TGVM**) in each 1-Mb window is 0.037%. For each iteration, the TGVM within each window was calculated by multiplying the SNP effects by each individual's SNP genotypes, summing across all SNPs in that window, and calculating the variance across all individuals (Wolc et al., 2012). The proportion of variance explained by the window was calculated by dividing the window variance by the variance across all markers in the genome. Windows with variances greater than expected for greater than 80% of the

iterations were considered the most probable in harboring a QTL and declared significant (Wolc et al., 2012). Additional windows of interest were defined as any non-significant window of the ten windows explaining the greatest proportion of TGVM for each analysis.

Under the hypothesis that SNP located in adjacent windows explaining large proportions of the total genetic variance were doing so because of linkage disequilibrium (**LD**) with a single QTL, these windows were combined into an extended window to estimate the total amount of genetic variance explained by that QTL. Specifically, the decision to combine windows was made if two adjacent or nearly adjacent windows were among the ten explaining the greatest proportion of TGVM for each analysis, and the window was extended beyond two Mb so that it was continuous and to include any other adjacent windows in the two percent of windows explaining the greatest proportion of TGVM for each analysis. As with 1-Mb windows, confidence that an extended window harbored a QTL was tested by considering whether or not it explained a greater than expected percent of the TGVM. To calculate the expected TGVM for these extended windows, the expected percentage of the TGVM for each 1-Mb window (0.037%) was multiplied by the number of 1-Mb windows that were combined. Estimates of the percentage of the TGVM of each extended window were generated using MCMC sampling with 120,000 iterations with every 100^{th} iteration of the last 100,000 iterations stored. As with 1-Mb windows, a threshold of 0.80 was used such that if greater than 80% of the iterations generated a percentage of the TGVM greater than expected for the extended window, the region was defined as significant and harboring a QTL.

Identification of Candidate Genes

Positional candidate genes that may harbor mutations underlying the genetic variance in windows with greatest percentage of the TGVM were explored using the NCBI genome database

(http://www.ncbi.nlm.nih.gov/genome/) and BioMart (www.ensembl.org). Focus was on genes located in significant regions or 2-Mb up and downstream of the significant 1-Mb windows as recommended based on simulation (Garrick and Fernando, 2013) or within the extended window. Prior evidence of QTL near or in significant 1-Mb, extended windows, or windows of interest was explored using Animal QTLdb (www.animalgenome.ofg/QTLdb/; Hu et al., 2016).

RESULTS AND DISCUSSION

Records from a total of 4,916 cows were used, and 826 of these cows contributed both primiparous and multiparous phenotypes (Table 1). On average, multiparous cows had greater DMI, MBW, and MilkE compared to primiparous cows (Table 2). The range in RFI of multiparous cows was approximately twice as great as that of primiparous cows.

Genetic Parameters

Feed efficiency is a complex trait (an outcome) that is influenced by multiple underlying traits, including DMI, milk production, and maintenance energy requirements. Heritability estimates for DMI, MBW, and MilkE in primiparous and multiparous cows ranged from 0.20 to 0.51 (Table 3), which is within the range of estimates previously established for these traits (for example, see Veerkamp, 1998; 2012). Our research also establishes a significant genetic component for RFI with heritability estimates ranging from 0.13 to 0.18 based on the current genomic analyses (Table 3) and traditional pedigree (Tempelman et al., 2015). Identifying and understanding the function of biological pathways underlying this genetic regulation of RFI could aid in the development of genetic, management, or nutritional strategies to improve feed efficiency in dairy herds. However, a challenge in understanding this genetic architecture is that RFI appears to be a truly multigenic trait that is influenced by many genes, each having a relatively small effect (Verbyla et al., 2010; Pryce et al., 2012; Yao et al., 2013). Thus, it is

important to minimize non-genetic factors that may compromise the ability to identify specific genomic regions of importance. In the current study, we analyzed data separately for primiparous and multiparous cows because of potential physiological differences between parities that could influence the RFI phenotype. Most notably, primiparous cows typically continue to grow in frame throughout their first lactation (Perotto et al., 1992) and this may impact the utilization of energy in primiparous compared to multiparous cows. It is quite notable that the range of RFI phenotypes was greater for multiparous cows compared to primiparous cows in the current study, resulting in very different estimates of genetic variance for primiparous and multiparous cows. Using the majority of the same cows but pedigree relationships and a different modelling strategy, Lu et al. (2017) also generated numerically larger estimates of genetic variance for multiparous cows. However, unlike the present study, estimates of residual variance in multiparous cows were nearly three times estimates in primiparous cows, leading to a much greater heritability estimate in primiparous cows (0.39) than in multiparous cows (0.22). Additionally, the genetic correlation between RFI in primiparous and RFI in multiparous cows was less than 1 (Table 3), further supporting that the underlying genetic variation differs in part between primiparous versus multiparous cows.

Genome-wide Association Study for RFI

The GWAS demonstrated that even though the regulation of RFI includes a genetic component, this regulation is highly polygenic with no individual region explaining a large proportion of the total genetic variation. All GWAS converged. In primiparous cows, the 1-Mb window with the greatest TGVM was located at 1 Mb on BTA 12 (Table 4), while in multiparous cows the window with the greatest TGVM was found at 33 Mb on BTA 28 (Table 5). No single window was considered statistically significant for either primiparous or

multiparous cows (Table S1 and S2). However, in primiparous cows, multiple windows in the region of 31 Mb through 38 Mb on BTA 27 were identified as regions of interest, while multiple windows in the region of 93 to 96 Mb on BTA 4 were regions of interest for multiparous cows. Therefore, adjacent windows in these regions were combined into extended windows to determine if they explained a greater than expected proportion of the TGVM. Together, the extended windows on BTA 27 explained 2.13% of the TGVM, and 95.3% of these iterations had a greater TGVM than expected for the region (Table 6). Thus a significant QTL for RFI in primiparous cows resides in the region of 31 to 38 Mb on BTA 27 (Supplemental Figure S1). In multiparous cows, the extended region on chromosome 4 explained 1.5% of TGVM and 79.5% of the iterations explained greater than the expected proportion of TGVM.

The significant QTL on BTA 27 has previously been associated with variation in DMI in primiparous cows (Veerkamp et al., 2012) and harbors multiple genes (Table 7). Among the genes in this region, the gene that encodes the beta-3 adrenergic receptor (***ADRB3***), beginning at 32.9 Mb, is particularly intriguing as a candidate gene for RFI because of the important role for beta adrenergic receptors in the mobilization and utilization of energy. In particular, agonists of the beta-adrenergic receptors have long been recognized as repartitioning agents that promote growth efficiency in meat animals (Etherton and Smith, 1991), although their role in lactating animals remains largely undefined. The identification of a significant QTL for RFI that includes the ADRB3 gene, combined with evidence that this gene is expressed in bovine adipose (Sumner and McNamara, 2007) and mammary (Inderwies et al., 2003) tissues identify *ADRB3* as a novel positional candidate gene for future investigation of physiological pathways underlying genetic differences for RFI in lactating Holstein cows.

The extended window on BTA 4 fell just short of reaching the significance threshold utilized in this study. Nevertheless, among the genes harbored within this region of BTA 4 is the gene that encodes the hormone leptin (***LEP***), starting at 93.2 Mb. Leptin is produced in adipose tissue, proportionally to mass, and functions in part to maintain energy balance by regulating appetite (Barb et al., 2006; Henry et al., 1999). Leptin signals through the central nervous system to elicit changes in feeding behavior, metabolism and endocrine physiology (Frühbeck et al., 1998) and also stimulates lipolysis through autocrine or paracrine effects on adipocytes (Frühbeck et al., 1997, 1998; Siegrist-Kaiser et al., 1997). Expression of this gene has previously been associated with variation in RFI in dairy cattle (Xi et al., 2015). Comparing mRNA levels in serum samples of cows with low versus high RFI, these authors found that *LEP*, and other genes in leptin-neuropeptide Y signaling pathway, were down-regulated in low RFI cows, suggesting that this pathway may affect feed efficiency. In the current study, the 1-Mb window on BTA 4 beginning at 95 Mb was also identified as a region of interest for DMI in multiparous cows while variants in *LEP* have previously been associated with variation in feed intake and energy balance albeit in primiparous dairy cattle (Liefers et al., 2002, 2005; Banos et al., 2008).

Prior studies that identified QTL for RFI were primarily focused on RFI in growing dairy cattle or beef steers, and studies of relatively small populations of lactating mature cows (Table 8). An earlier analysis using novel methodology and a subset of data used in the current study identified 188 SNP s associated with RFI (Yao et al., 2013). Only one region of the 10 most significant regions reported by Yao et al. (2013) and the current study were in common. However, Yao et al. (2013) and the current study each identified a region on BTA 11 that fell within the same confidence interval identified in beef cattle (Sherman et al., 2009). Many of the

significant or most explanatory regions were unique across studies. This observation further supports the conclusion of the current study that RFI is a highly polygenic trait, and may suggest that the identification of QTL influencing RFI is highly sensitive to specific populations, statistical approaches, and definition of RFI studied.

Using data from 527 primiparous cows, Verbyla et al., (2010) predicted that there are 472 QTL for energy balance, which is mathematically equivalent to RFI (Veerkamp, 1998). With only 527 phenotypes, power was not high enough to be able to detect significant QTL, but the authors suggested with more phenotypes, GWAS could lead to identification of possible candidate genes related to energy balance. As such, we used 4,916 cows in the present study. However, the improvements in power were limited by dividing the records into primiparous and multiparous groups and the lower heritability estimated in this study than in Verbyla et al. (2010).

Genome-wide Association Study for Underlying Traits

Similar to RFI, convergence was achieved and only a small proportion of genetic variance was explained by any single 1-Mb window for DMI, MilkE, or MBW in primiparous (Figure 1) or multiparous (Figure 2) cows. In primiparous cows, there were 7 significant windows across the 4 traits, including 3 windows for DMI (BTA 10, 25, and 26) and 4 windows for MBW (BTA 4, 5, 6, and 18). The region surrounding 105 Mb on BTA 5 has previously been identified as a QTL for body size traits in beef cattle (Saatchi et al., 2014b), and the window on BTA 6 was also a region of interest for MilkE in primiparous cows.

In multiparous cows, 4 windows (BTA 14, 18, 22, and 28) were considered significant and all were associated with MBW (Table 5). The gene-rich region on BTA 18 (Table 9) was previously identified in the United States dairy cattle population as related to body size traits. A

SNP in this window, ss86324977, had the greatest probability of a non-zero effect on MBW in the present study and was previously identified as explaining the most variation for body depth, sire and daughter calving ease, sire and daughter stillbirth, rump width, stature, and strength (Cole et al., 2009). Cole et al. (2009) identified this SNP as located in an intron of the sialic acid binding IG-like lectin (***Siglec***)-5 gene, which has been shown to be linked to a leptin deficiency that may cause a delay in parturition, and consequently, larger calf size. The region identified on BTA 14 has been associated with body weight traits in beef cattle (Saatchi et al., 2014b), and the region on BTA 28, has been identified as a QTL for birth weight in Angus cattle (McClure et al., 2010).

In addition to the significant QTL defined by the 1-Mb windows, extended windows were investigated for 2 additional regions associated with MBW. In primiparous cows, windows beginning at 102 and 103 Mb on BTA 3 were combined. This extended window explained 1.05 percent of TGVM and 78.6% of iterations explained greater variance than expected. For multiparous cows, the 1-Mb windows on BTA 7 from 92 to 93 Mb were combined. This extended window explained 1.59 percent of TGVM and 92.2% of iterations explained greater variance than expected. Although only the extended region on BTA 7 achieved statistical significance as defined for this study, both regions have previously been associated with BW traits in cattle. The extended region on BTA 3 was previously identified as a QTL for body size related traits, including calf size and calving ease in Charolais cattle (Purfield et al., 2015). Likewise, the extended region on BTA 7 was identified in previous studies in beef cattle for body-size related traits. This region has been significantly associated with birth weight, weaning weight, yearling weight, mature weight, and rib eye area across multiple breeds of beef cattle (Snelling et al., 2010; Saatchi et al., 2014b; Weng et al., 2016).

373 ***Pleiotropic or closely linked regions***. Overlapping or nearby windows of interest for
374 multiple traits were explored because of the possibility of a pleiotropic QTL causing genetic
375 variation in multiple traits. Regions in common between DMI and RFI but not between DMI
376 with MilkE or MBW were of particular interest because of the possibility that genetic variation
377 here could be exploited to reduce DMI without adversely impacting MilkE or MBW. Three such
378 regions were identified on BTA 12 and BTA 18 in primiparous cows and on BTA 4 in
379 multiparous cows. Additional regions that may characterize pleiotropic effects on multiple traits
380 include the window on BTA 6 that was a region of interest for MilkE and MBW in primiparous
381 cows; BTA 13 from 43 to 46 Mb that was a region of interest for MilkE and RFI in multiparous
382 cows; and BTA 28 from 20 to 33 that included regions of interest for all traits evaluated in
383 multiparous cows.

384 Pleiotropy is not surprising in light of the genetic correlations between these traits. Using
385 nearly 2,000 US cows and more than 2,000 cows from the Netherlands, up to half of which were
386 in common with the current study, genetic correlations of 0.70 (the Netherlands) and 0.89 (US)
387 were estimated between RFI and DMI (Manzanilla-Pech et al., 2016). In both the US and Dutch
388 population of cows, genetic correlations were estimated at 0.63 between DMI and MilkE and at
389 0.56 in the Netherlands and 0.46 in the US populations between DMI and MBW.

390 **CONCLUSIONS**
391
392 This study characterized the genetic architecture of RFI and related traits of DMI, milk
393 energy and MBW. In general, these traits are highly polygenic with no individual region
394 explaining large proportions of the total genetic variation. Furthermore, the genetic basis of
395 these traits is not static throughout the life of the dairy cow as indicated by moderate genetic
396 correlations between primiparous and multiparous cows. Nevertheless, 2 noteworthy QTL were

identified; in primiparous cows, a significant QTL was identified for RFI on BTA 27 that harbors the positional candidate gene *ADRB3*, and the region of BTA 4 that harbors the gene encoding LEP was identified as a region of interest for RFI and DMI in multiparous cows. Overall, these results illustrate the physiological complexity underlying the genetic regulation of feed efficiency in lactating dairy cattle.

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Table 1. Distribution of cow records with phenotype and genotype data by parity and location.

	Primiparous	Multiparous	Total number of unique cows[1]
United States	1,916	1,843	3,309
Canada	213	112	220
Netherlands	581	372	937
United Kingdom	365	340	450
Total	3,075	2,667	4,916

[1] Difference between sum of primiparous and multiparous records and total number of unique cows is the number of cows contributing to both primiparous and multiparous records.

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Table 2. Means ± SD and *(minimum, maximum)* for selected traits for primiparous (N=3075) and multiparous (N = 2667) cows: residual feed intake (RFI), DMI, metabolic body weight (MBW), milk energy (MilkE), milk yield (MY), percentage fat in milk, percentage protein in milk, change in BW (ΔBW), and DIM.

	Primiparous			Multiparous		
RFI (kg)	0.00	± 1.30	*(-7.31, 5.47)*	0.01	± 1.79	*(-13.81, 17.96)*
DMI (kg/d)	20.63	± 3.23	*(9.16, 32.68)*	25.23	± 4.47	*(11.07, 44.72)*
MBW (kg)	114.6	± 8.34	*(61.6, 155.3)*	129.5	± 10.01	*(94.22, 170.1*
MilkE (Mcal/d)	24.82	± 4.54	*(6.18, 39.45)*	32.14	± 5.83	*(10.06, 52.74)*
MY (kg/d)	35.38	± 6.74	*(8.34, 56.51)*	46.35	± 8.72	*(13.73, 77.66)*
Fat (%)	3.69	± 0.50	*(1.85, 5.59)*	3.66	± 0.57	*(1.83, 6.45)*
Protein (%)	3.00	± 0.28	*(2.28, 4.11)*	2.96	± 0.32	*(2.01, 4.70)*
ΔBW (kg/d)	0.40	± 0.47	*(-5.21, 3.19)*	0.29	± 0.66	*(-5.30, 4.83)*
DIM	85.01	± 28.67	*(61, 186)*	89.28	± 30.38	*(63, 185)*

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Table 3. Estimates (SE) of phenotypic (r_P), and genetic (r_g) correlation, additive genetic variance (σ_a^2) and heritability for residual feed intake (RFI), DMI, metabolic body weight (MBW), and milk energy (MilkE) for primiparous and multiparous cows.

	r_P	r_g	σ^2_a		h^2	
			Primiparous	Multiparous	Primiparous	Multiparous
RFI	0.27 (0.03)	0.76 (0.13)	0.23 (0.05)	0.41 (0.09)	0.14 (0.03)	0.13 (0.03)
DMI	0.49 (0.03)	0.78 (0.07)	1.22 (0.14)	1.61 (0.24)	0.32 (0.03)	0.23 (0.03)
MBW	0.78 (0.01)	0.92 (0.03)	24.7 (1.83)	33.5 (2.86)	0.51 (0.03)	0.46 (0.03)
MilkE	0.49 (0.03)	0.61 (0.08)	3.18 (0.35)	3.44 (0.53)	0.31 (0.03)	0.20 (0.03)

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Table 4. Location, percentage of total genetic variance explained, and rank of the ten 1-megabase (Mb) windows that explained the most genetic variation in primiparous cows for each trait DMI, residual feed intake (RFI), milk energy (MilkE), and metabolic body weight (MBW). Results are based on Bayes B analysis with 1% of SNP included in the model and starting parameters based on Bayes C with all SNP included in the model.

Chromosome	Mb[1]	Percentage[2]	RFI[3]	DMI	MilkE	MBW
1	52	0.69	3			
2	33	0.31	7			
3	102	0.55				9
3	103	0.82				5
4	7	0.58		10		
4	14	1.35				2*
5	105	2.03				1*
5	117	0.28	9			
5	118	0.63		7		
6	88	1.15,0.79			1	6*
7	18	0.49			9	
7	91	0.84				4
8	76	0.52			8	
9	84	0.46			10	
10	33	1.87		2*		
11	76	0.79	2			
12	1	1.09	1			
12	20	0.58		8		
12	25	0.60	5			
13	69	0.57				8
17	30	0.72		5		
18	5	0.55			5	
18	23	1.15				3*
18	64	0.28	8			
18	65	0.80		4		
19	38	0.47		9		
21	2	0.59			4	
21	12	0.53			7	
22	1	0.53				10
22	37	0.60			3	
23	3	1.39			6	
23	39	0.27	10			
23	47	0.68		6		
25	30	0.96		3*		
26	32	1.89		1*		

Table 4 (continued)

27	32	0.63	4		
27	33	0.44	6		
28	15	0.84		2	
X	132	0.68			7

[1]Distance in Megabases to the start of the window

[2]Percentage of the total genetic variance explained by the window. x,x reflects the traits in the order of the columns from left to right.

[3]Rank is based on the total genetic variance explained by the window with rank = 1 denoting the window explaining the greatest percentage of total genetic variance explained for that trait.

*In greater than 80% of iterations, the variance was greater than expected (0.37%)

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Table 5. Location, percentage of total genetic variance explained, and rank of the ten 1-megabase (Mb) windows that explained the most genetic variation in multiparous cows for each trait DMI, residual feed intake (RFI), milk energy (MilkE), and metabolic body weight (MBW). Results are based on Bayes B analysis with 1% of SNP included in the model and starting parameters based on Bayes C with all SNP included in the model.

Chromosome	Mb[1]	Percentage[2]	RFI[3]	DMI	MilkE	MBW
2	44	0.41		7		
2	53	0.95				5
3	114	0.38		9		
4	93	0.67	2			
4	95	0.48, 0.55	4	5		
5	67	0.64		4		
6	60	1.23		1		
7	18	0.39			6	
7	27	0.29		8		
7	92	0.82				7
7	93	0.83				6
9	95	0.51			7	
11	51	0.37	7			
11	13	0.35			10	
11	66	0.73		2		
11	105	0.37		6		
13	43	0.34	8			
13	46	0.66			1	
14	11	0.68				9
14	20	1.24				2*
18	23	0.66				10
18	57	1.08				4*
19	51	0.32	9			
20	27	0.39			4	
20	48	0.86			3	
21	16	0.44	6			
21	25	0.31	10			
21	63	0.73				8
22	1	1.19				3*
24	54	0.64		3		
25	13	0.67	3			
26	28	0.47	5			
26	39	0.47			8	
26	45	0.46			2	
28	20	1.62				1*

Table 5 (continued)

28	24	0.43			9
28	26	0.30		9	
28	33	0.80	1		
X[4]	30	0.56			5

[1]Distance in Megabases to the start of the window

[2]Percentage of the total genetic variance explained by the window. x,x reflects the traits in the order of the columns from left to right.

[3]Rank is based on the total genetic variance explained by the window with rank = 1 denoting the window explaining the greatest percentage of total genetic variance explained for that trait.

[4]X refers to the X-specific portion of the X chromosome

*In greater than 80% of iterations, the variance was greater than expected (0.37%)

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Table 6. Percentage of genetic variance explained and the corresponding percentage of iterations in which the variance was greater than expected for windows extended beyond 1 megabase (Mb).

Parity[1]	Trait[2]	BTA	Position, Mb[3]	Percent[4]	Iterations (%)
1	MBW	3	102-103	1.05	78.6
	RFI	27	31-38	2.13	95.3
2	MBW	7	92-93	1.59	92.2
	RFI	4	93-96	1.50	79.5

[1]1, primiparous cows; 2, multiparous cows.
[2]MBW = metabolic body weight; RFI = residual feed intake.
[3]Location in Megabases of the window.
[4]Percentage of the total genetic variance explained by the window.

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Table 7. Candidate protein-coding genes in windows extended beyond 1 megabase (Mb).

Parity[1]	Trait	BTA	Position, Mb	Candidate Genes
1	MBW	3	102-103	*ARTN, ATP6VOB, B4GALT2, CCDC24, DMAP1, DPH2, ERI3,IPO13, KDM4A, PTPRF, RNF220, ST3GAL3*
	RFI	27	31-38	*ADAM2, ADAM9, ADAM18, ADAM32, ADGRA2, ADRB3, AP3M2, ASH2L, BAG4, BRF2, C8orf4, CHRNA6, CHRNB3, CSGALNACT1, DDHD2, DKK4, EIF4EBP1, ERLIN2, FGFR1, FNTA, GINS4, GOLGA7, GOT1L1, GPAT4, HGSNAT, HOOK3, HTRA4, IDO1, IDO2, INTS10, IKBK, KAT6A, KCNU1, LETM2, LSM1, PLAT, PLEKHA2, PLP5, POMK, PROSC, PSD3, RAB11FIP11, RNF170, SFRP1, SH2D4A, SLC20A2, SMIM19, STAR, TACC1, THAP1, TM2D2, UNC5D,WHSC1L1, ZMAT4, ZNF703*
2	MBW	7	92-93	*ADGRV1, ARRDC3, CETN3, LYSMD3, MBLAC2, POLR3G*
	RFI	4	93-96	*AHCYL2, CALU, CCDC136, CEP41, COPG2, CPA1, CPA4, CPA5, FAM71F1, FLNC, IMPDH1, IRF5, KCP, KLHDC10, LEP, LRRC4, MEST, MKLN1, NRF1, OPN1SW, PLXNA4, PODXL, PRRT4, RBM28, SMO, SND1, SSMEM1, STRIP2, TMTM209, TNPO3, TSGA13, TSPAN33, UBE2H, ZC1HC1*

[1]1, primiparous cows; 2, multiparous cows.

Table 8. Locations in the bovine genome identified in previous genome-wide association studies as associated with RFI in beef or dairy cattle.

Reference[1]	N	Breed	Age Group	Location[2]
Nkrumah et al., 2007	400	Multiple beef	Steers	**1:61*** , 5:70*, **7:9*** , 8:40*, 12:37*, 14:51*, 16:17*, 17:19*, 26:25*, 29:7-28*
Marquez et al., 2009	218	Multiple beef	Steers and heifers	2:126*, 6:55*, 7:93*, 10:31*, 11:29*, 13:18*, 16:43*
Sherman et al., 2009	400	Multiple beef	Steers	**1:0-3*** , 3:52*, **7:5-26*** , **11:3-16*** , **18:17-35*** , **19:15-44*** , 19:59*, 22:9-16*, 23:21-31*, 26:32-35*
Bolormaa et al., 2011	379	Angus	Steers and heifers	2:24*, 2:63*, 3:105*, 4:41*, 4:91*, 5:110*, 7:102*, 8:86*, 8:93*, 10:18*, 20:33*
Bolormaa et al., 2011	852	Multiple beef	Steers	2:22*, 5:51*, 8:90*, 9:14*, 9:60*, 11:1*, 12:55*, 17:43*, 18:3*, 25:12*, 27:21*
Rolf et al., 2012	698	Angus	Steers	1:130*, 2:31*, 2:45*, 2:76*, 8:6*, 8:110*, 11:70*, 12:72*, 17:4*, 28:14*
Pryce et al., 2012	1,782	Holstein	Heifers	14:25, 14:36
Yao et al., 2013	402	Holstein	Multiple lactations	1:146*, 7:50*, **11:5*, 11:6*** , 8:11*, 12:78*, 18:56*, **19:29*** , 22:38*, **26:28***
Serao et al., 2013	976	Angus and Simmental	Steers	4:75*, 5:60*, 6:109*, 8:108*, 17:28*, 17:59*, 22:57*, 24:2*
Lu et al., 2013	751	Multiple beef	Growing males and females	**1:2*** , **1:61*** , 1:157*, 3:102*, 7:27*, 10:91*, 10:95*, 16:27*, 20:44*, 24:29*
Saatchi et al., 2014a	5,133	Multiple beef	Steers and heifers	6:50*, 10:58*, 14:41*, 14:43*, 15:82*, **18:22*** , 18:37*, 19:54*, 20:4*, 25:7*
Tolkamp et al., 2014	1,804	Holstein-Fresian	Primiparous	5:6*, 5:87*, 8:113*, 21:68*, 26:29*
Santana et al., 2014	720	Nellore	Young bulls and steers	8:4*, 21:71*

Table 8 (continued)

Olivieri et al, 2016	896	Nellore	Growing males and females	1:100, 1:121, 4:105, 4:118, 7:92, 8:41, 8:103, 10:68, 18:11, 21:18, 24:59
Santana et al., 2016	1334	Nellore	Young bulls and steers	2:43*, 3:2*, 5:101*, 15:62*, 22:48*
Present study	2,667	Holstein	Multiparous	4:93, 4:95, 4:96, 6:128, **11:12**, 21:16, 25:13, 19:51, **26:28**, 28:33
Present study	3,075	Holstein	Primiparous	1:52, 11:76, 12:1, 12:25, 18:64, 23:39, 25:0, 25:2, 27:32, 27:37

*Significance threshold set in the original study is met

[1]The trait considered in Tolkamp et al. (2014) was energy balance

[2] The ten most significant locations, or in the absences of significance criteria, locations explaining the greatest proportion of genetic variance are provided.Format is Chromosome:Megabase (Mb) where the Mb may be a range (x – x) encompassing a confidence window. Results reported in centiMorgans were converted to Mb using an alignment to Baylor cattle SNPs provided by AnimalQTLdb. Locations published as SNP were converted to the whole Mb lying upstream of the SNP using the NCBI SNP database. Regions in bold are in common between 2 or more studies.

Table 9. Candidate protein-coding genes within 2 megabases (Mb) of significant 1-Mb windows for traits underlying feed efficiency.

Parity[1]	Trait	BTA	Position, Mb[2]	Candidate Genes
1	DMI	10	33	*BMF, BUB1B, C15orf51, DPH6, EIF2AK4, FAM98B, FSIP1, MEIS2, RASGRP1, SRP14, SPRED1, THBS1, TMCO5*
		25	30	*AUTS2, CALN1, CHCD2, CRCP, GUSB, NUPR2, KCTD7, PHKG1, RABGEF1, SBDS, SUMF2, TMEM248, TPST1, TYW1, WBSCR17*
		26	32	*ACSL5, ADD3, ADRB1, CASP7, CCDC186, DCLRE1A, DUSP5, GPAM, HABP2, MXI1, NHLRC2, NRAP, PDCD4, PLEKHS1, RBM20, SHOC2, SMC3, SMNDC1, TDRD1, TECTB, XPNPEP1, ZDHHC6*
	MBW	4	14	*ASB4, ASNS, C1GALT1, COL28A1, DLX5, DLX6, DYNC1I1, MIOS, PDK4, PON1, PON2, PON3, PPP1R9A, RPA3, RPL7, SDHAF3, SLC25A13, TAC1*
		5	105	*ACRBP, AKAP3, ANO2, ATN1, B4GALNT3, C12orf57, C1RL, C1S, CCDC77, CCND2, CD4, CD9, CD27, CDCA3, CHD4, CLSTN3, COPS7A, DDX11, DYRK4, ENO2, FGF6, FGF23, FKBP4, FOXM1, GALNT8, GNB3, GPR162, IFFO1, ING4, IQSEC3, ITFG2, KCNA1, KCNA5, KCNA6, KDM5A, LAG3, LPAR5, LPCAT3, LRRC23, LTBR, MLF2, MRPL51, NCAPD2, NINJ2, NOP2, NRIP2, NTF3, PARP11, P3H3, PEX5, PHB2, PIANP PLEKHG6, PRMT8, PTMS, PTPN6, RBP5, RHNO1, SCNN1A, SLC6A12, SLC6A13, SPSB2, TAPBPL, TEAD4, TIGAR, TNFRSF1A, TPI1, TSPAN9, TSPAN11, TULP3, USP5, VAMP1, VWF, ZNF384*
		6	88	*ADAMTS3, AFM, AFP, ALB, AMBN, AMTN, ANKRD17, CABS1, COX18,CSN1S1, CSN2, CSN3, DCK, ENAM, EPGN, GC, GRSF-1, IL-8, JCHAIN, MOB1B, MTHFD2L, NPFFR2, ODAM, PPBP, RASSF6, RUFY3, SLC4A4, SULT1B1, SULT1E1, UGT2A3, UTP3*
		18	23	*ADGRG3, ADGRG5, AKTIP, AMFR, ARL2BP, BBS2, CCDC102A, CCL17, CCL22, CES5A, CHD9, CIAPIN1, CNGB1, COQ9, CPNE2, CX3CL1, DOK4, DRC7, FAM192A, FTO, GNAO1, HERPUD1, IRX3, IRX5, IRX6, KATNB1, KIFC3, LPCAT2, MMP2, MT3, MT4, NLRC5, NUDT21, NUP93, OGFOD1, PLLP,*

Table 9 (continued)

				POLR2C, RBL2, RPGRIP1L, RSPRY1, SLC12A3, SLC6A2, TEPP, TOX3, USB1, ZNF319
2	MBW	14	20	*ATAD2, DERL1, EFCAB1, FAM83A, HAS2, PCMTD1, PRKDC, SNAI2, SNTG1, SPIDR, ST18, TBC1D31, UBE2V2, WDYHV1, ZHX1, ZHX2*
		18	57	*ACPT, AKT1S1, ALDH16A1, AP2A1, ASPDH, ATF5, BAX, BCAT2, BCL2L12, CA11, C19orf68, C19orf81, CABP5, CCDC114, CCDC155, CD37, CEACAM18, CLEC11A, CPT1C, CRX, CYTH2, DBP, DHDH, DKKL1, EHD2, ELSPBP1, EMC10, EMP3, ETFB, FAM83E, FCGRT, FGF21, FLT3LG, FUT1, FUT2, FUZ, GLTSCR1, GLTSCR2, GRIN2D, GRWD1, GYS1, HAS1, HRC, HSD17B14, IGLON5, IL4I1, IRF3, IZUMO1, IZUMO2, JOSD2, KCNA7, KCNC3, KCNJ14, KDELR1, KLK1, KLK4, KLK5, KLK6, KLK7, KLK8, KLK10, KLK11, KLK12, KLK13, KLK14, LIG1. LIM2, LIN7B, LMTK3, LRRC4B, MAMSTR, MED25, MYBPC2, MYH14, NAPSA, NKG7, NOSIP, NR1H2, NTF4, NTN5, NUCB1, PIH1D1, PLEKHA4, PNKP, POLD1, PPFIA3, PPP1R15A, PPP2R1A, PRR12, PRRG2, PTH2, PTOV1, RCN3, RASIP1, RPL18, RRAS, RUVBL2, SCAF1, SEPW1, SHANK1, SIGLELC1, SLC17A7, SLC6A16, SNRNP70, SPACA4, SPACA6, SPHK2, SULT2B1, SYNGR4, SYT3, TBC1D17, TEAD2, TMEM143, TRPM4, TSKS, TULP2, VN1R4, VRK3, VSIG10L, ZNF114, ZNF175, ZNF432, ZNF473, ZNF613, ZNF614*
		22	1	*AZI2, CMC1, DBNL, EGFR, EOMES, LANCL2, MRPS24, NEK10, PGAM2, RBMS3, SEC61G, SLC4A7, UBE2D4, URGCP, VOPP1*
		28	20	*ADO, ARID5B, CTNNA3, EGR2, JMJD1C, NRBF2, REEP3, RTKN2, ZNF365*

[1]1, primiparous cows; 2, multiparous cows.
[2]Significance declared when in greater than 80% of iterations, the variance was greater than expected (0.37%)

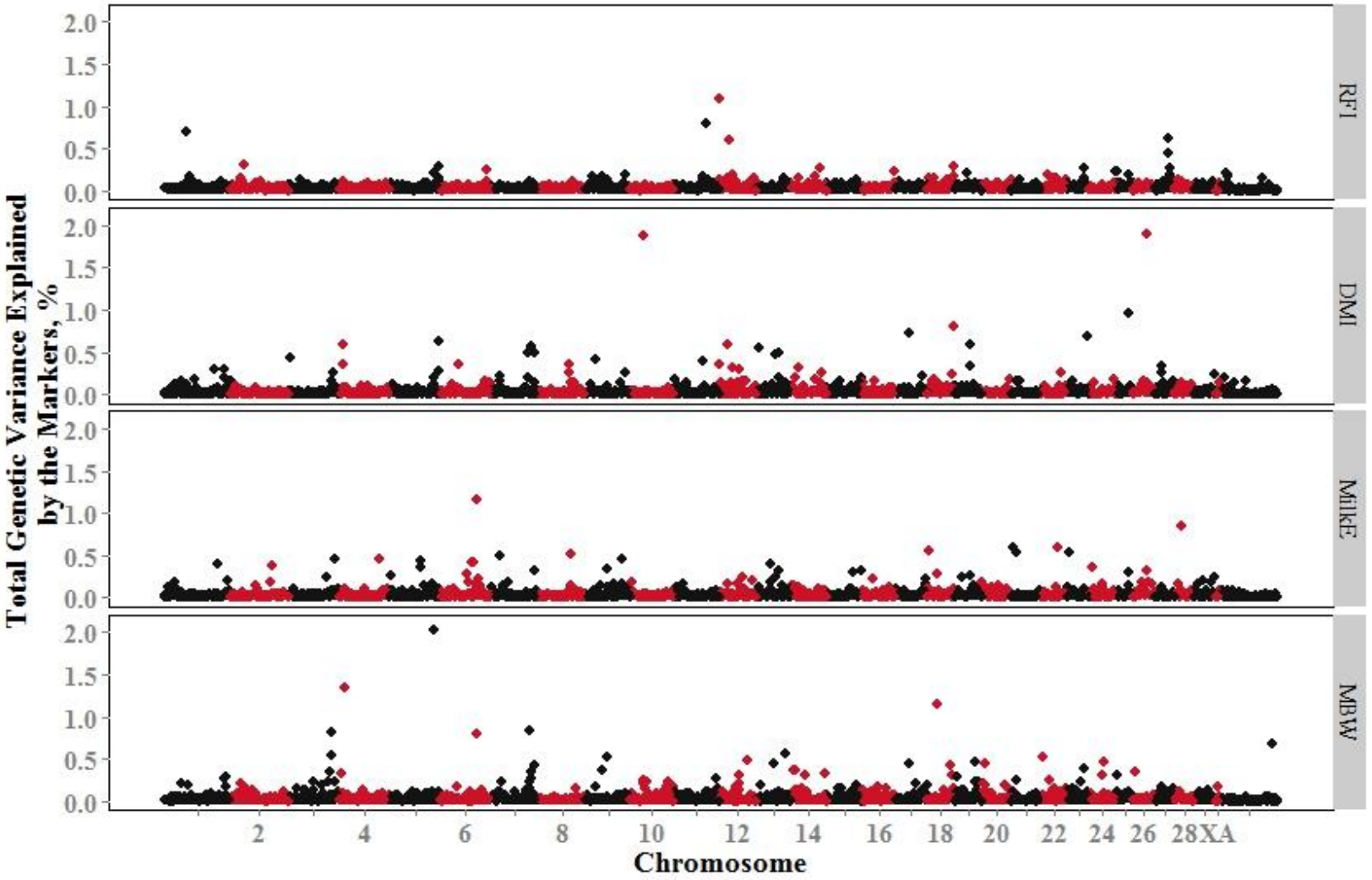


Figure 1. Manhattan plots of 1-Mb windows for residual feed intake (RFI), DMI, and the energy sinks milk energy (MilkE) and metabolic body weight (MBW) in primiparous cows. Chromosomal location XA refers to the pseudo autosomal portion of the X chromosome with the X-specific markers the set of black markers at the right edge of the plots.

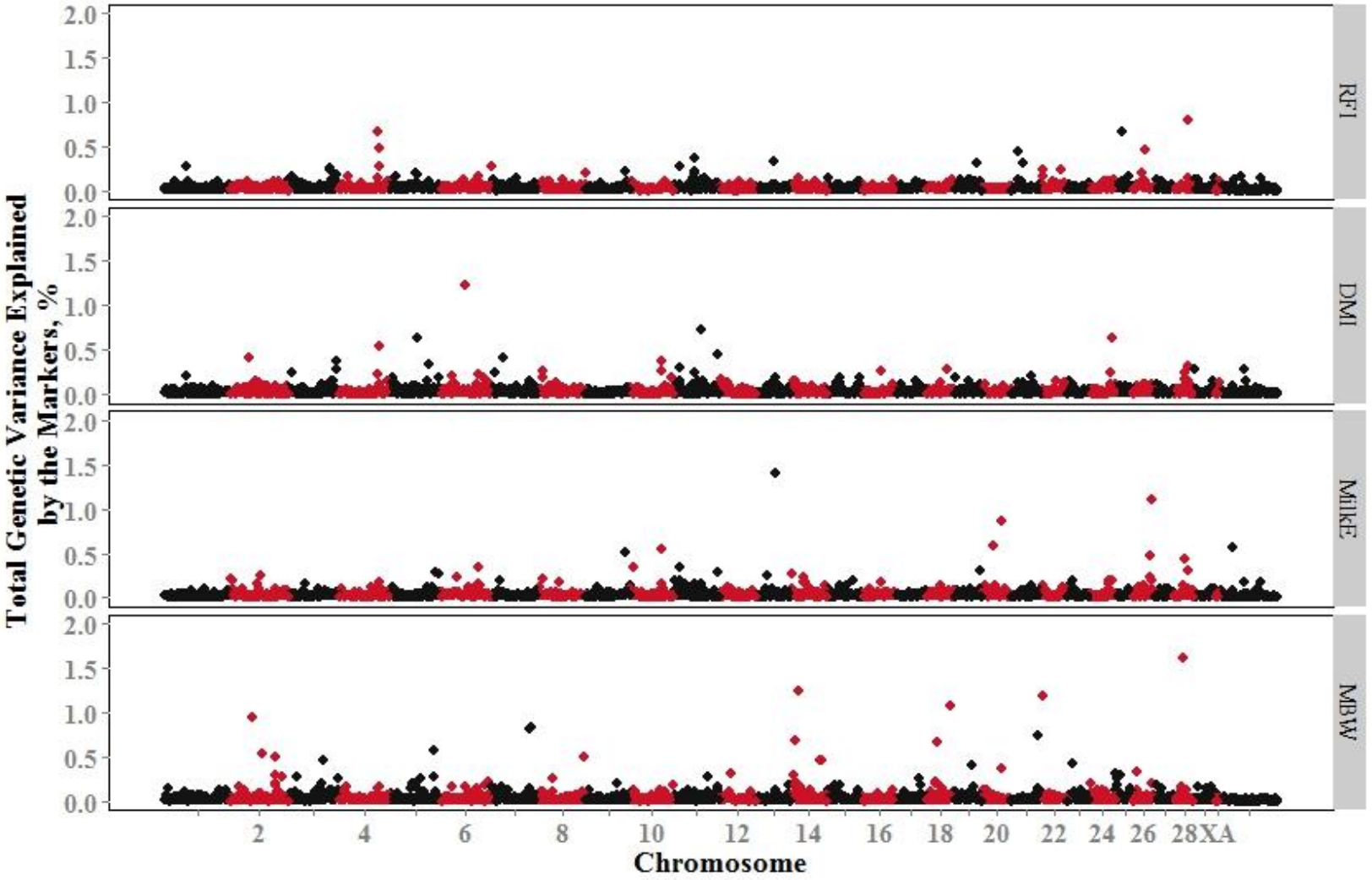
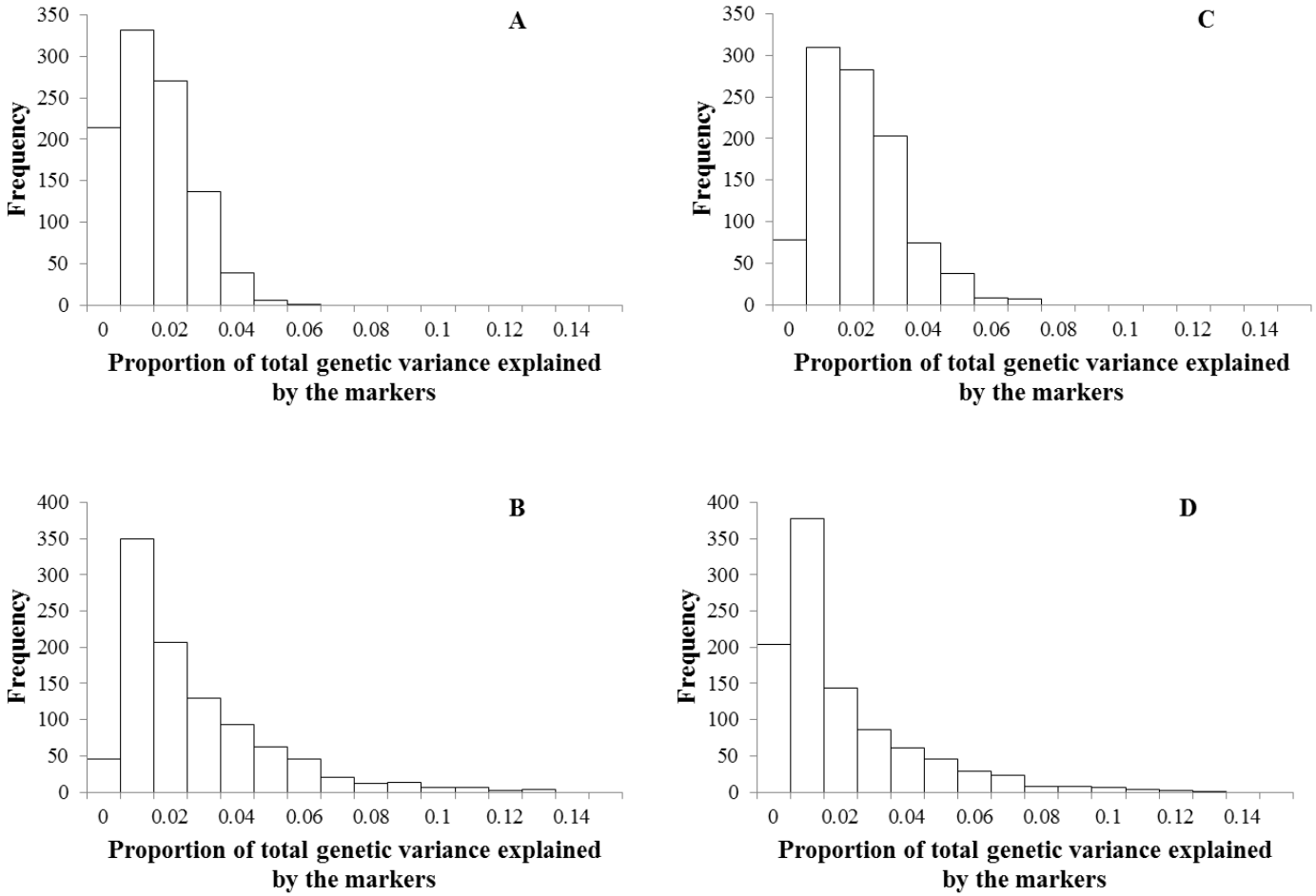


Figure 2. Manhattan plots of 1-Mb windows for residual feed intake (RFI), DMI, and the energy sinks milk energy (MilkE) and metabolic body weight (MBW) in multiparous cows. Chromosomal location XA refers to the pseudo autosomal portion of the X chromosome with the X-specific markers the set of black markers at the right edge of the plots.

Supplementary Tables and Figures



Supplemental Figure S1. Distribution of genetic variance for each of 999 iterations for extended windows spanning from A) 102 through 103 megabases (Mb) on BTA 3 for metabolic body weight (MBW) in primiparous cows, B) 31 through 38 Mb on BTA 27 for residual feed intake (RFI) in primiparous cows, C) 92 through 93Mb on BTA7 for MBW in multiparous cows and D) 92 through 95 on BTA 4 for RFI in multiparous cows. Labels for the x-axis denote the maximum value included in the corresponding bar. Expectations were 0.074%, 0.296%, 0.074%, and 0.148% for panels A through D, respectively.

Supplemental Table S1. Ten 1-Mb windows with the greatest percentage of the total genetic variance explained by the markers for each DMI, metabolic body weight (MBW), residual feed intake (RFI), and milk energy (MilkE) in primiparous cows.

Trait	Chromosome[1]	Location (Mb)	No. SNP	%Var	Iterations (%)[2]
RFI	12	1	14	1.09	0.32
RFI	11	76	21	0.79	0.28
RFI	1	52	28	0.69	0.33
RFI	27	32	27	0.63	0.35
RFI	12	25	23	0.60	0.31
RFI	27	33	23	0.44	0.29
RFI	2	33	21	0.31	0.23
RFI	18	64	43	0.28	0.34
RFI	5	117	24	0.28	0.23
RFI	23	39	36	0.27	0.32
RFI	27	37	28	0.27	0.27
RFI	14	70	26	0.27	0.25
RFI	6	113	30	0.25	0.24
RFI	25	2	37	0.24	0.29
RFI	16	77	30	0.24	0.26
RFI	25	0	39	0.23	0.31
RFI	25	4	36	0.23	0.27
RFI	5	106	28	0.22	0.36
RFI	19	29	31	0.21	0.28
RFI	X	13	14	0.21	0.18
DMI	26	32	29	1.89	0.94
DMI	10	33	17	1.87	0.93
DMI	25	30	28	0.96	0.85
DMI	18	65	48	0.80	0.76
DMI	17	30	22	0.72	0.64
DMI	23	47	34	0.68	0.72
DMI	5	118	47	0.63	0.69
DMI	12	20	22	0.58	0.58
DMI	19	38	24	0.58	0.55
DMI	4	7	34	0.58	0.62
DMI	7	94	20	0.57	0.60
DMI	13	6	18	0.54	0.54
DMI	7	85	27	0.49	0.52
DMI	13	54	18	0.49	0.53
DMI	7	102	16	0.49	0.58
DMI	13	46	30	0.47	0.63
DMI	3	5	28	0.43	0.57
DMI	9	23	28	0.42	0.54
DMI	11	68	20	0.39	0.49
DMI	12	1	14	0.36	0.46
MilkE	6	88	36	1.15	0.83
MilkE	28	15	26	0.84	0.78

Supplemental Table S1 (continued)

MilkE	22	37	20	0.60	0.63
MilkE	21	2	18	0.59	0.75
MilkE	18	5	26	0.55	0.63
MilkE	23	3	26	0.54	0.55
MilkE	21	12	31	0.53	0.65
MilkE	8	76	24	0.52	0.56
MilkE	7	18	31	0.49	0.57
MilkE	9	84	16	0.46	0.46
MilkE	3	112	35	0.45	0.62
MilkE	4	95	22	0.45	0.52
MilkE	5	75	25	0.43	0.48
MilkE	6	74	25	0.42	0.45
MilkE	6	78	14	0.41	0.44
MilkE	1	126	23	0.39	0.47
MilkE	13	34	11	0.39	0.41
MilkE	2	98	20	0.38	0.44
MilkE	24	7	27	0.37	0.50
MilkE	5	74	23	0.37	0.45
MBW	5	105	29	2.03	1.00
MBW	4	14	19	1.35	0.95
MBW	18	23	34	1.15	0.94
MBW	7	91	32	0.84	0.68
MBW	3	103	26	0.82	0.65
MBW	6	88	36	0.79	0.88
MBW	X	132	15	0.68	0.69
MBW	13	69	24	0.57	0.79
MBW	3	102	26	0.55	0.57
MBW	22	1	27	0.53	0.81
MBW	9	48	16	0.53	0.64
MBW	12	68	32	0.49	0.67
MBW	24	34	29	0.47	0.56
MBW	19	48	30	0.47	0.56
MBW	17	33	25	0.45	0.62
MBW	13	41	31	0.44	0.68
MBW	20	9	30	0.44	0.62
MBW	18	57	25	0.42	0.62
MBW	7	102	16	0.42	0.60
MBW	23	39	36	0.39	0.65

[1] X refers to the X-specific portion of the X chromosome.

[2] Percentage of iterations in which the variance was greater than expected (0.37%).

Supplemental Table S2. Ten 1-Mb windows with the greatest percentage of the total genetic variance explained by the markers for each DMI, metabolic body weight (MBW), residual feed intake (RFI), and milk energy (MilkE) in multiparous cows.

Trait	Chromosome[1]	Location (Mb)	No. SNP	%Var	Iterations (%)[2]
RFI	28	33	30	0.80	0.38
RFI	4	93	24	0.67	0.32
RFI	25	13	24	0.67	0.30
RFI	4	95	22	0.48	0.28
RFI	26	28	22	0.47	0.26
RFI	21	16	25	0.44	0.33
RFI	11	51	11	0.37	0.21
RFI	13	43	16	0.34	0.20
RFI	19	51	24	0.32	0.29
RFI	21	25	21	0.31	0.22
RFI	4	96	27	0.28	0.27
RFI	6	128	24	0.28	0.22
RFI	11	12	31	0.28	0.26
RFI	1	53	20	0.27	0.24
RFI	3	100	24	0.26	0.23
RFI	3	99	24	0.24	0.22
RFI	22	48	27	0.24	0.27
RFI	22	2	27	0.23	0.22
RFI	11	49	25	0.22	0.22
RFI	9	93	23	0.22	0.19
DMI	6	60	18	1.23	0.64
DMI	11	66	24	0.73	0.50
DMI	24	54	17	0.64	0.47
DMI	5	67	16	0.64	0.48
DMI	4	95	22	0.55	0.55
DMI	11	105	30	0.44	0.47
DMI	2	44	16	0.41	0.39
DMI	7	27	25	0.41	0.41
DMI	3	114	30	0.38	0.45
DMI	10	75	13	0.37	0.33
DMI	5	93	20	0.33	0.37
DMI	28	32	28	0.32	0.37
DMI	11	12	31	0.30	0.39
DMI	18	49	25	0.29	0.39
DMI	X	59	16	0.29	0.30
DMI	3	115	23	0.28	0.34
DMI	29	3	26	0.28	0.39
DMI	8	9	26	0.27	0.37
DMI	16	44	10	0.27	0.37
DMI	10	74	25	0.26	0.37
MilkE	13	46	30	1.41	0.78
MilkE	26	45	35	1.10	0.76

Supplemental Table S2 (continued)

MilkE	20	48	15	0.86	0.49
MilkE	20	27	30	0.58	0.53
MilkE	X	30	17	0.56	0.46
MilkE	10	75	13	0.54	0.39
MilkE	9	95	27	0.51	0.49
MilkE	26	39	26	0.47	0.46
MilkE	28	24	24	0.43	0.48
MilkE	11	13	34	0.35	0.45
MilkE	10	6	23	0.34	0.44
MilkE	6	90	22	0.34	0.40
MilkE	19	60	51	0.31	0.52
MilkE	28	33	30	0.30	0.40
MilkE	5	110	26	0.28	0.40
MilkE	11	105	30	0.28	0.38
MilkE	14	4	47	0.26	0.49
MilkE	5	118	47	0.26	0.49
MilkE	13	25	26	0.25	0.34
MilkE	2	71	22	0.25	0.34
MBW	28	20	23	1.62	0.89
MBW	14	20	28	1.24	0.85
MBW	22	1	27	1.19	0.84
MBW	18	57	25	1.08	0.82
MBW	2	53	28	0.95	0.71
MBW	7	93	23	0.83	0.71
MBW	7	92	24	0.82	0.74
MBW	21	63	27	0.73	0.62
MBW	14	11	33	0.68	0.65
MBW	18	23	34	0.66	0.61
MBW	5	105	29	0.58	0.67
MBW	2	74	19	0.53	0.54
MBW	2	109	31	0.50	0.58
MBW	8	109	28	0.49	0.56
MBW	3	82	19	0.47	0.51
MBW	14	72	21	0.46	0.56
MBW	14	71	21	0.46	0.49
MBW	23	12	24	0.42	0.57
MBW	19	42	32	0.41	0.54
MBW	20	46	21	0.36	0.47

[1] X refers to the X-specific portion of the X chromosome.

[2] Percentage of iterations in which the variance was greater than expected (0.37%).